US-PAT-NO: 6350580

DOCUMENT-IDENTIFIER: US 6350580 B1

TITLE: Methods for detection of a target nucleic acid using a probe comprising secondary structure

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A safety pin probe, as utilized in the present invention, requires a "universal" hairpin probe 1 (FIG. 9, b171), comprising a hairpin structure, with a fluorophore (FAM) on the 5' arm of the hairpin and a quencher (Dabcyl) on the 3' arm, and a probe 2 (FIG. 9, SP170a) comprising a stem-loop comprising two domains: the 5' two thirds of probe 2 have a (universal) sequence complementary to the hairpin probe 1, and nucleotides that will stop the DNA polymerase, and the 3' one third of probe 2, which serves as the target specific primer. As the polymerase, primed from the reverse primer (that is, the 3' one third of probe 2) synthesizes the top strand, the 5' end of probe 2 will be displaced and degraded by the 5' exonucleolytic activity until the "stop nucleotides" are reached. At this time the remainder of probe 2 opens up or unfolds and serves as a target for hairpin probe 1, thereby separating the fluorophore from the quencher (FIG. 9).

| L Number | Hits | Search Text | DB | Time stamp |
|---|---|---|---|---|
| 1 | 7 | primer near5 hybridi$6 near5 stem | USPAT | 2003/01/07 06:40 |
| 2 | 9 | primer near9 hybridi$6 near9 stem | USPAT | 2003/01/07 06:40 |
| 3 | 2 | (primer near9 hybridi$6 near9 stem) not (primer near5 hybridi$6 near5 stem) | USPAT | 2003/01/07 06:45 |
| 4 | 10 | (loop or stem) near11 (universal) same primer | USPAT | 2003/01/07 06:46 |

| L Number | Hits | Search Text | DB | Time stamp |
|---|---|---|---|---|
| 1 | 26 | "6027893" "5843650" "5451503" "6235889" | USPAT | 2003/01/07 17:43 |
| 2 | 8 | ("6027893" "5843650" "5451503" "6235889") and (polyethylene or glycol or polypropylene or polyamide or polyester$) | USPAT | 2003/01/07 18:23 |
| 3 | 4555 | primer same (polyethylene or glycol or polypropylene or polyamide or polyester$) | USPAT | 2003/01/07 17:47 |
| 4 | 234 | primer same (link$7) same (polyethylene or glycol or polypropylene or polyamide or polyester$) | USPAT | 2003/01/07 17:49 |
| 5 | 6 | primer same (link$7) same (polyethylene or glycol or polypropylene or polyamide or polyester$) same amplif$8 | USPAT | 2003/01/07 17:52 |
| 6 | 105 | primer same (link$7) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$) | USPAT | 2003/01/07 17:55 |
| 7 | 189 | (primer$2 or oligo$9 or probe$2) same (link$7) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$4) and amplif$9 | USPAT | 2003/01/07 17:56 |
| 8 | 83 | (primer$2 or oligo$9 or probe$2) near9 (link$7) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$4) and amplif$9 | USPAT | 2003/01/07 18:18 |
| 9 | 7105 | (branch$6) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$4) | USPAT | 2003/01/07 18:57 |
| 10 | 554 | ((branch$6) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$4) ) and (amplif$7 or detect$5) and (DNA or RNA or nucleic or oligo$9) | USPAT | 2003/01/07 18:19 |
| 11 | 59 | (((branch$6) near9 (polyethylene or glycol or polypropylene or polyamide or polyester$4) ) same (DNA or RNA or nucleic or oligo$9)) and (amplif$7 or detect$5) | USPAT | 2003/01/07 18:20 |
| 12 | 22125 | (linker$2 or polyethylene or glycol or polypropylene or polyamide or polyester$) near9 (stab$9 or steric or hindrance or enahnc$9) | USPAT | 2003/01/07 18:25 |
| 13 | 21 | ((linker$2 or polyethylene or glycol or polypropylene or polyamide or polyester$) near9 (stab$9 or steric or hindrance or enahnc$9)) same (amplif$8) | USPAT | 2003/01/07 18:33 |
| 14 | 301 | "flexible linker" | USPAT | 2003/01/07 18:33 |
| 15 | 116 | "flexible linker" same (nucleic or RNA or DNA or oligo$8 or primer$2 or probe$) | USPAT | 2003/01/07 18:47 |
| 16 | 28 | egholm-$.in. | USPAT | 2003/01/07 18:50 |
| 17 | 8 | egholm-$.in. and flexible | USPAT | 2003/01/07 18:54 |
| 18 | 8 | egholm-$.in. and flexible | USPAT | 2003/01/07 18:50 |
| 19 | 1 | egholm-$.in. and flexible | DERWENT | 2003/01/07 18:50 |
| 20 | 1 | 2002-140094.NRAN. | DERWENT | 2003/01/07 18:51 |
| 21 | 20 | "5281701" | USPAT | 2003/01/07 18:54 |
| 22 | 10869 | (branch$6) near5 (polyethylene or glycol or polypropylene or polyamide or polyester$4 or link$7) | USPAT | 2003/01/07 18:58 |
| 23 | 26 | (branch$6) near5 (polyethylene or glycol or polypropylene or polyamide or polyester$4 or link$7) near8 (probe$4 or primer$4) | USPAT | 2003/01/07 18:59 |

| L Number | Hits | Search Text | DB | Time stamp |
|---|---|---|---|---|
| 1 | 1 | "6197556" | USPAT | 2003/01/07 14:32 |
| 2 | 1 | "6197556" and RNA | USPAT | 2003/01/07 15:02 |
| 3 | 1 | "6197556" and linker | USPAT | 2003/01/07 15:03 |
| 4 | 13 | "5451503" and linker | USPAT | 2003/01/07 15:03 |
| 5 | 3 | "5451503" and flexible and linker | USPAT | 2003/01/07 15:09 |
| 6 | 52 | linker same flexible same (glycol or poyethylene or polypropylene or polyamide$ or polyester) | USPAT | 2003/01/07 15:10 |

| L Number | Hits | Search Text | DB | Time stamp |
|---|---|---|---|---|
| 1 | 927 | "5114869" "4965188" "5573905" "5624798" "5627032" "5663062" | USPAT | 2003/01/07 09:05 |
| 2 | 66 | "5114869" "5573905" "5624798" "5627032" "5663062" | USPAT | 2003/01/07 09:06 |
| 3 | 69 | "5114839" "5573905" "5624798" "5627032" "5663062" | USPAT | 2003/01/07 09:15 |
| 4 | 1 | "6451588" | USPAT | 2003/01/07 09:18 |
| 5 | 22 | "5527675" | USPAT | 2003/01/07 09:23 |
| 6 | 3 | "6140490" | USPAT | 2003/01/07 09:23 |
| 7 | 2 | "6255476" | USPAT | 2003/01/07 09:23 |
| 8 | 0 | 9801628.0 | DERWENT | 2003/01/07 09:23 |
| 9 | 9 | "9801628" | DERWENT | 2003/01/07 09:24 |
| 10 | 2 | "9937806" | DERWENT | 2003/01/07 09:25 |
| 11 | 1 | "5674683" | DERWENT | 2003/01/07 09:38 |